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16. ABSTRACT

An extensive review on methodology for the determination of arsenic and its compounds. The review includes collection and storage of samples, sample preparation for a) determination of total arsenic content and b) of specific arsenic compounds. A section covers standards for the accuracy of analysis. Methods of measurement described are colorimetry, atomic absorption spectrometry, radioactivation analysis, other spectroscopic methods, chromatography, and electromechanical methods.

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JOURNAL ARTICLE

Determination of arsenic and arsenicals in foods and other biological materials

By

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Contents

I. Introduction ---- 123
II. Collection and storage of samples ---- 124
III. Sample preparation ---- 126
a) For determination of total arsenic content ---- 126
1. Destruction of biological matrix ---- 127
2. Recovery of arsenic from ashed or digested samples ---- 129
b) For determination of specific arsenic compounds ---- 131
IV. Standards ---- 132
V. Methods of measurement ---- 133
a) Colorimetry ---- 133
b) Atomic absorption spectrometry ---- 134
c) Radioactivation analysis ---- 137
d) Other spectroscopic methods ---- 138
e) Chromatography ---- 140
f) Electrochemical methods ---- 142
Summary ---- 143
References ---- 144

I. Introduction

Arsenic residues in food and in the tissues of man and animals have long been recognized as indicative of wide-spread environmental contamination by arsenicals. Although appreciable quantities of arsenic are introduced into the atmosphere through smelting operations, the principal cause of pollution of the general environment is the use of arsenicals for crop protection. Inorganic arsenicals have been used as pesticides for over 250 years, the first published references for which date to 1681 (see ROBBINS *et al.* 1942). In modern times, organic, as well as inorganic, arsenic compounds have been and continue to be used as insecticides, herbicides, and fungicides. While there was a steady decline in arsenical

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use in the United States until the early 1970s, demand has recently increased because of restrictions on the use of persistent organochlorine pesticides. In 1973 the agricultural industry consumed over 80% of all arsenic compounds used in the United States (*U.S. Department of Interior* 1975).

Organoarsenicals are also popular as animal feed additives. Such compounds are added to poultry and swine feeds in the United States and other countries for growth stimulation and disease control. They are usually added in amounts from ten to 20 μg/g. Consequently, poultry, eggs, and pork contain higher arsenic levels than beef and dairy products.

Applications of arsenicals to plant foliage and soils leave residues which are ultimately consumed by man directly or indirectly through the food chain. Arsenic residues are common in foods, although regulated by law in most countries to levels of less than 0.5 to 2 μg/g (ROWE 1973). Residues in fruits and vegetables as high as several μg/g have been reported in recent years (GEISMAN *et al.* 1969, STEEVENS *et al.* 1972). Livestock forage crops often contain even higher levels. Meat and poultry products sold in the United States usually contain less than one μg/g of arsenic (CORNELIUSSEN 1969, MORRISON and GEORGE, 1969); however, fish and other marine animals are more efficient at the bioaccumulations of arsenic and thus commonly contain one to ten μg/g of arsenic and sometimes considerably more (LUNDE 1968 and 1970, UTHE *et al.* 1974). Primarily through dietary exposure, man has accumulated residues of 0.001 to three μg/g in his body tissues, blood, hair, and nails (SMITH 1967). Meat, fish, poultry, and dairy products accounted for 78.7% of the dietary intake of arsenic in the United States during fiscal year 1973 (MAHAFFEY *et al.* 1975). The total quantity of arsenic ingested was estimated to correspond to 10.1 μg/day.

Analytical methodology for the determination of arsenic in biological matrices does not differ substantially from that required for other substrates. In most cases, however, it is necessary to destroy the organic substrate prior to the determinative step. This may be accomplished through digestion or ashing. Most procedures then call for oxidation of the arsenic compounds remaining in the residue to the arsenite form and subsequent reduction to arsine gas (AsH_3). This hydride may be measured directly or trapped in an appropriate sorbent for later analysis. The determinative step may be by colorimetry, atomic absorption spectroscopy, neutron activation analysis, or any of several other methods.

II. Collection and storage of samples

For foodstuffs and animal feeds, official guidelines have been established in the United States for the collection of samples for residue analyses. Instructions on the collection of food and feed samples for pesticide residue analysis (including arsenicals) are given in the U.S. Food and Drug Administration's (FDA) Inspection Operation Manual and Compliance Program Guidelines Manual. Total composite samples

of approximately 20 lb (nine kg) are collected from retail outlets, warehouses, and transport vehicles. Additional specifications are given for sample sizes for selective samples of raw agricultural products including milk, eggs, and meat. The FDA also conducts "Market Basket" surveys 30 times each yr in each of the four major geographic regions of the United States (MAHAFFEY *et al.* 1975). Some 117 food items which constitute the average diet of teenage males are collected and analyzed as composites of 12 food classes for pesticides and trace metals.

General rules for preparing and compositing food and feed samples are given in Sections 140 to 142 of the FDA Pesticide Analytical Manual (MCMAHON 1975). Since most tolerances have been established for whole raw agricultural commodities, it may be desirable in many cases to analyze the total sample without regard to inedible portions. For some raw food commodities Regulation 40 CFR 180.1 (j) specifies which portions are to be taken for analysis. When only edible portions are of interest, discretion is left to the analyst, although guidelines are provided for several foodstuffs in Section 141.12 of the FDA Pesticide Analytical Manual. Soil, waxes, and decomposed parts should be removed prior to analysis. For processed foods, packing liquors are analyzed only if normally consumed. Dehydrated or concentrated foods are not reconstituted unless special preparation is specified. Frozen foods are thawed and drained and remaining edible portions only are prepared for analysis.

Arsenic residues are quite often higher in certain portions of plants than in others. Levels in inedible stems, pods, roots, peels, and rinds are sometimes higher than those in the edible portions. For example, arsenic residues in potato peelings have been found to be up to 138 times higher than those for the whole tuber (STEEVENS *et al.* 1972). The highest arsenic levels in treated tomatoes (GEISMAN *et al.* 1969), blueberries (ANASTASIA and KENDER 1973), and kale (JONES and HATCH 1945) appear to be in the roots. Similarly, ambient arsenic concentrations seem to be highest in roots of trees (ANDREN *et al.* 1973). On the other hand, residues are more uniformly distributed in bean and pea plants (JONES and HATCH 1945) and in apples (STANKIEWICZ *et al.* 1963). Care should be exercised, therefore, in the selection of plant parts for analysis. Even for edible portions, normal cooking procedures may alter the arsenic content. Water in cooking the food and juices lost in the process need not be analyzed if they are not normally consumed.

Most foods and other biological specimens are not homogeneous and are too large for an entire sample to be analyzed. In the case of food, samples must be taken that are representative of the whole item or of the entire portion. It may be necessary to take several portions and homogenize them together in a high-speed blender prior to further treatment for analysis. For investigations into the exposure of animals and human beings, of course, it will be desirable to analyze different organs, body parts, and excreta separately. In human beings, arsenic levels are usually highest in hair and nails.

As for any sample taken for analysis of trace residues, special care

should be taken to prevent contamination. For total arsenic determinations, contamination problems are somewhat less than those associated with trace organic analyses. If intact organoarsenical pesticides (e.g., methyl- and phenylarsonic acids) are to be determined by some non-specific technique such as electron-capture gas chromatography, the usual precautions against sample contamination by organic compounds should be exercised. Thus, scrupulously-cleaned glass containers with polyfluorocarbon-lined lids should be used for collection, and sample handling with vinyl or rubber gloves should be minimized or avoided. On the other hand, glass bottles and jars may be a source of sample contamination in the case of total arsenic analysis, since soda-lime glass may contain 500 μg/g of arsenic (*National Bureau of Standards* 1973). Quite the opposite problem was reported by PORTMANN and RILEY (1964), however, who found that 16% of radio-labelled arsenic in seawater samples stored in soft glass bottles was lost in 16 days due to adsorption on the container walls. Storage in polyethylene bottles resulted in six % loss in ten days. Containers made of plastics which incorporate little or no plastizers, catalysis residues, or other additives are safest (e.g., Teflon®, Tedlar®, and laboratory-grade polyethylene). Wherever possible, samples should be handled with stainless steel implements. Disposable scalpels should be avoided. Blood should be taken with glass syringes fitted with stainless-steel needles and transferred to borosilicate glass vials with polyfluorocarbon-lined caps.

Since most biological materials will decompose readily, samples should be stored frozen, or at least refrigerated. Lyophilization is not generally recommended as some arsenic compounds may be lost through volatilization. Effects of prolonged storage on recoveries of organoarsenic compounds should be assessed on an individual case basis.

III. Sample preparation

a) For determination of total arsenic content

Sample treatment prior to analysis depends largely on the analytical technique utilized. If the total sample is to be analyzed, homogenization may be necessary. For foods and human tissues this is normally accomplished in a Waring® Blendor, Duall® grinder, or Hobart® mixer, usually with anhydrous sodium sulfate added to absorb moisture. Instructions for homogenization are given in Volume 1 of the FDA Pesticide Analytical Manual (MCMAHON 1975) and in the analytical manual for human and environmental samples published by the U.S. Environmental Protection Agency (THOMPSON 1974). The portion of the total sample taken for homogenization should be large enough to be representative of the whole sample. Raw produce which contains free juices (e.g., tomatoes, oranges, apples) and which is too large to be homogenized whole should be first frozen and then diced while still in the frozen state.

Final preparation of the homogeneous sample or aliquot may involve destruction of the organic matrix or extraction of the compound of interest. For determination of the total arsenic content of solid biological samples, wet or dry ashing is usually required. In this process the organic substrate is destroyed and all arsenicals are converted to inorganic forms.

1. Destruction of biological matrix.—

a) Wet ashing.—Wet ashing, or digestion, is accomplished through treatment (usually under heat) of the biological material with strong mineral acids, peroxides, or perchlorates. The degree of rigor required in the digestion process depends on the nature of the substrate. If strongly oxidizing conditions are maintained, all forms of arsenic should be oxidized to As(V). Less oxidizing conditions, however, may result in the formation of As(III) compounds, some of which have substantial vapor pressures. For example, $AsCl_3$ (bp 130°C) and As_2O_3 (sublimes 193°C) are frequently produced by the process of wet ashing and may be lost if precautions are not taken. One mechanism which has been employed to prevent the formation of volatile $AsCl_3$ during digestion is the addition of molybdenum (VI) (Christian and Feldman (1970) or vanadium (V) (Uthe *et al.* 1974) compounds as catalysts. On the other hand, mild digestion of some biological material (e.g., urine) with 10 *N* or greater HCl will allow $AsCl_3$ to be extracted with benzene for determination (Simon *et al.* 1968).

Treatment with 4*N* HCl for 30 min has been reported to be efficient for determination of arsenic in liver, kidney, and muscle (Kingsley and Schaffert 1951). Prolonged digestion with HNO_3 at 180°C has been used for plant tissues and animal muscle with apparent high recovery of arsenic (Portmann and Riley 1964). Other strong oxidizing mixtures which have been used for biological tissues are perchloric acid (Chu *et al.* 1972); "Caro's acid," which is made from concentrated H_2SO_4 and 50% H_2O_2 (Samsahl 1967), and "chromic acid," made from sodium dichromate and concentrated H_2SO_4 (Bouchard 1973). High reagent blanks are always possible with any mineral acid. The probability of such contamination is substantially increased when chromic acid is used. The chromic acid oxidation, however, offers the advantage that it is carried out at room temperature and is very rapid. Otherwise digestion is most often conducted under reflux and requires 30 min to several hr.

Because of the wide variety of substrates which are encompassed by foods and other plant and animal tissues, fluids, and excreta, no single wet-ashing procedure may be adequate for all biological samples. Christian and Feldman (1970), however, feel that wet digestion in 3:1:1 nitric, sulfuric, and perchloric acids is the most generally useful. It will attack all but the most resistant substrates and is sufficiently strongly oxidizing to minimize losses of volatile elements.

Highly fatty samples cannot be sufficiently ashed by wet methods. HCl, H_2SO_4, and HNO_3 will not fully oxidize materials which are high in lipid content, while $HClO_4$ oxidation may be so exothermic as to result

in explosion. Foaming is also a problem with fat samples (STONE 1967). Some success with wet digestion of high-lipid materials has been reported by FELDMAN (1974), but dry ashing is usually best for fatty foods and animal tissues.

β) Dry ashing.—Dry ashing involves the decomposition of the substrate material by combustion in air or oxygen, sometimes with the aid of chemical oxidants. Conventional dry-ashing methods generally involve temperatures of 400 to 700°C and should only be utilized with special precautions for the determination of arsenic because of the probability of losses through volatilization. Recovery of arsenic from biological material by dry ashing has been reported to be satisfactory when magnesium nitrate is added (EVANS and BANDEMER 1954). By this method STONE (1967) reported 95% recovery of arsenic at levels of 0.1 ppm and below for various animal tissues and fluids. GEORGE *et al.* (1973) modified the method by using a 1:1 mixture of MgO and $Mg(NO_3)_2$. Samples of swine liver, kidney, and skin weighing ten g were heated with six g of the mixture at 555°C in a muffle furnace for two hr. In a collaborative study involving eight laboratories, recoveries of arsenic were 98.8 to 103.4% (overall coefficient of variation, 8.31%) at 0-, 1-, and 2-ppm concentrations. UTHE *et al.* (1974), however, obtained recoveries of only 89 to 95% for arsenic added to fish and shellfish as As_2O_3, cacodylic acid, and tetramethylarsonium hydroxide when they used the dry-ashing method of GEORGE *et al.* (1973), while 99.6 to 103% recoveries were realized using a vanadium pentoxide-catalyzed, H_2SO_4-HNO_3 digestion procedure.

Fusion with alkali metals can, in a manner similar to that of combustion with $Mg(NO_3)_2$, reduce losses by volatilization. A large excess of the alkali salt or hydroxide is utilized and, hence, may afford serious problems with respect to the introduction of As or interfering contaminants into the blanks. BOWEN (1968) claimed a 96% recovery of arsenic from plant leaves by heating for a few min at 390°C with an equimolar mixture of $NaNO_3$ and KNO_3.

Combustion in an oxygen-rich atmosphere may allow reduction of ashing temperatures and lower losses of volatile arsenic compounds. Normally the reaction is carried out in a closed system, such as a Parr bomb or the Schöniger flask. Only small samples can be combusted in this manner and the material must be thoroughly dried before burning. Freeze-drying is often utilized for this purpose, so that any gains made towards reducing losses on combustion may be offset by losses during the drying process. SCHWEDT and RÜSSEL (1972), however, did report quantitative recovery of arsenic from biological material when they used the Schöniger-flask technique.

A variation of the oxygen-combustion method, which appears to offer the maximum advantage to the analyst concerned with the determination of arsenic and other volatile, as well as nonvolatile, elements in a wide variety of organic matrices, is low-temperature ashing (LTA). The

method, first reported by GLEIT and HOLLAND (1962), involves the production of an atmosphere of highly reactive ionized oxygen (a plasma) by means of microwave-induction. Combustion can be accomplished at temperatures of under 100°C at rates on the order of one g/hr. The ashing rate is a function of the total surface area of the specimen and its mineral content. The usual tissue sample should not require more than three hr/g (GLEIT 1965), although several days may be necessary to ash completely some materials such as bone and dense wood samples. Quantitative recovery of arsenic from blood (added as $HAsO_2$) was reported after 1.5 hr of ashing by LTA, as opposed to 23% recovery from 24-hr ashing at 400°C and 0% after three-hr ashing at 900°C in a muffle furnace (GLEIT and HOLLAND 1962). TARUMOTO and FREISER (1975), on the other hand, found 25% less arsenic in a human lung tissue sample after overnight ashing in an LTA than they determined by wet digestion. The apparent loss was considered acceptable, however, in view of the long time required for wet ashing. Commercial LTA instruments are now available from two sources in the United States. Their principal disadvantage is high cost ($6,000.00 to $10,000.00).

2. Recovery of arsenic from ashed or digested samples.—Since the arsenic content of the ashed or digested residue will probably be low in comparison to many other elements, it is necessary to isolate and concentrate the arsenic for measurement. This may be accomplished by solvent extraction or volatilization techniques.

After digestion or ashing, the inorganic residue is commonly dissolved in water. Arsenic can then be removed selectively from the solution by partitioning into an organic solvent containing a chelating agent or by co-precipitation with a metal hydroxide. The most useful chelating agents for arsenic are diethylammonium diethyldithiocarbamate (DDDC) and ammonium pyrrolidine dithiocarbamate (APDC). These agents are selective for As(III), so that any As(V) present must be reduced prior to extraction. The selectivity can be of value, however. If all the arsenic is first oxidized to the pentavalent state, DDDC or APDC can be used to extract several potentially interfering metals before reduction of the arsenic to the trivalent state (TALMI and FELDMAN 1975). Also, it may be of interest in some cases to determine separately the more toxic arsenite forms present, although arsenic appears to exist more in organic forms in many biological tissues. (REINKE *et al.* 1975, LAKSO and PEOPLES 1975). These chelating agents are soluble in chloroform and methyl isobutyl ketone (MIBK), which can be evaporated for concentration of the arsenic to enhance analytical sensitivity. The aqueous solutions may require acidification prior to extraction. The pH of the solution can also be adjusted to introduce some selectivity into the extraction process (CHRISTIAN and FELDMAN 1970). VELEKER (1962) used DDDC in chloroform to isolate arsenic from antimony and bismuth down to 0.06 μg/g concentration.

The solubilities of arsenic halides in certain organic solvents likewise

offer a facile route for arsenic isolation through extraction. For example, aliphatic ketones may be used to extract $AsCl_3$ from aqueous solutions highly acidified with HCl. 2-Butanone, 2-pentanone, or 2-heptanone mixed in a 3:2 ratio with CCl_4 have been used successfully for this purpose (GAGLIARDI and WÖSS 1969).

Co-precipitation with thionalide (glycolic-β-aminonaphthalide) has been employed to recover arsenic and other V A and VI A metaloids from solution (PORTMANN and RILEY 1964); however, only As(III) salts can be removed in this manner. The technique is particularly valuable when perchlorate digestion is used, as it affords a means of separating arsenic from $HClO_4$ (TALMI and NORVELL 1975).

Co-precipitation with $BaSO_4$ favors removal of pentavalent arsenic from solution, and may be used to differentiate between As(III) and As(V) concentrations (KAR and SINGH 1968). Background difficulties from the use of inorganic compounds may be intolerable, however, especially for ultratrace analysis.

The halides and hydride of trivalent arsenic are volatile and thus may be separated from substrates by vaporization. $AsCl_3$ may be evolved from the sample by treatment of the ash with HCl or during the wet digestion process (TALMI and FELDMAN 1975). Although less volatile, $AsBr_3$ (bp 221°C) has also been distilled from digested biological material using a special still (SANDELL 1959).

The most often used method of recovery of arsenic from samples is by conversion to the highly volatile hydride, AsH_3 (bp −55°C). Early methodology involved reduction with zinc dust in strong mineral acid, usually in the presence of stannous salts. A problem may be encountered if H_2S and other sulfur compounds are not first eliminated from the sample. Presumably, less volatile compounds such as As_2S_3 are formed. KINGSLEY and SCHAFFERT (1951) circumvented the problem by first digesting samples of animal tissue in an open flask with concentrated HCl for 15 min. This was said to be sufficient to volatilize H_2S and mercaptans from the sample prior to the preparation of AsH_3. Other reducing agents, such as $TiCl_3$ or alkali metal iodides, have been used (TALMI and FELDMAN 1975, TALMI and BOSTICK 1975 a), but sodium borohydride ($NaBH_4$) is found to be the most generally useful today (BRAMAN *et al.* 1972). A major advantage of borohydride reduction over Zn or $TiCl_3$ reduction is that the arsenic need not be reduced to the trivalent state prior to treatment (TALMI and BOSTICK 1975 a). However, BRAMAN *et al.* (1972) noted that it is necessary for arsenic to be in an oxidized state and for the solution to be made neutral or alkaline in order to avoid decomposition of the $NaBH_4$. This may be somewhat troublesome with digested samples which contain large quantities of strong acids. Both Cu^{+2} and Ag^{+} interfere with the generation of arsine (AsH_3) and must be removed before treatment with $NaBH_4$ (BRAMAN *et al.* 1972). SCHMIDT and ROYER (1973) found that the yield of arsine from digested sludge samples was five times higher by $NaBH_4$ reduction than by Zn reduction.

Analytical time was also reduced by a factor of 30. Interestingly, these authors carried out the reduction under acid conditions, employing solid pellets rather than a dilute solution of $NaBH_4$. FIORINO *et al.* (1976) used a base-stabilized solution of $NaBH_4$ in ten % NaOH to generate arsine from acid-oxidized food samples. Pre-reduction of As(V) to As(III) with ten % NaI was required since the arsine generation was carried out at a lower pH than usual.

Arsine generated by the several reduction reactions described above is usually collected in a reservoir for analysis. Balloons or plastic bags are often used for this purpose (FREEMAN and UTHE 1974, HOOVER *et al.* 1974). Other means of trapping the gas are by cold-trap condensation (HOLAK 1969, ORBEIM and BOVEE 1974, TALMI and BOSTICK 1975 b) or absorption into a reactive medium, such as sodium hypobromite (SANDELL 1959), silver diethyldithiocarbamate (HUNDLEY and UNDERWOOD 1970), or silver nitrate solution (MADSEN 1971). Often it is possible or desirable to distill the arsine directly into the instrument of detection or to generate it therein (BRAMAN *et al.* 1972, LICHTE and SKOGERBOE 1972, FIORINO *et al.* 1976, SIEMER *et al.* 1976). The latter procedure offers a distinct advantage in that arsine is reported to decompose even at room temperature (ROBINSON *et al.* 1974).

Other volatile or organic-soluble arsenic compounds, such as methylated arsenic and triphenylarsine, may also be prepared as routes of isolation; however, these compounds are rarely chosen except for determination of specific arsenicals.

b) For determination of specific arsenic compounds

There have been relatively few attempts to determine specific arsenic compounds as residues in biological materials. LUNDE (1973 a) found that he could separate organic from inorganic arsenic in marine algae by first extracting with a mixture of chloroform, methanol, and water (4:2:1) to remove lipids, boiling the residue in water to produce an aqueous extract, and subjecting the latter to anion-exchange chromatography to effect separation. He subsequently found that the majority of organoarsenic compounds present in marine organisms were stable to treatment with 6.6 *N* HCl at 100°C (LUNDE 1973 b). Under this treatment, trivalent inorganic arsenicals could be removed by distillation as $AsCl_3$.

BRAMAN and FOREBACK (1973) found that methylarsonic acid and dimethylarsinic acid (cacodylic acid) could be reduced to volatile methyl- and dimethylarsine, respectively, by sodium borohydride reduction at pH 1 to 2. Liquid nitrogen was used to trap the alkylarsines for analysis. Similarly, TALMI and BOSTICK (1975 b) carried out the same reduction, but used toluene chilled to −5°C to trap the alkylarsines. SODERQUIST *et al.* (1974) determined cacodylic acid in aqueous samples by conversion to iododimethylarsine, which could then be extracted into hexane for analysis. Cacodylic acid, monosodium methanearsonate (MSMA), and disodium methanearsonate (DSMA) can be converted to their ethylene

glycol esters for ease of extraction and individual determination by gas chromatography (JOHNSON *et al.* 1972).

Roxarsone (4-hydroxy-3-nitrobenzenearsonic acid), a drug used for poultry and swine, was extracted from feed premix with one % aqueous ammonium carbonate (FRAHM *et al.* 1975). GEORGE and MORRISON (1971) found that two % dibasic potassium phosphate was effective for extraction of roxarsone from poultry feeds. Water has been used to extract cacodylic acid, MSMA, sodium arsenate, and sodium arsenite from plant materials (SACHS *et al.* 1971). They could then be separated for determination by paper chromatography. DAUGHTREY *et al.* (1975) isolated mono- and dimethylarsine from urine by extraction with benzene and hexane, respectively, after treatment with KI.

Sodium arsenite and arsenate were isolated and determined along with the sodium salts of methylarsonic acid and cacodylic acid by EDWARDS *et al.* (1975) using electrophoresis. The compounds could be extracted from cellular material by boiling in water or water-methanol.

IV. Standards

In order to obtain acceptable accuracy in the analysis of arsenic and arsenicals in biological materials, reliable standards must be available. Primary standards are needed for calibration of instrumentation and for determination of recoveries of arsenic from the substrate. The only certified primary standard available is the National Bureau of Standards' SRM 83C, which is 99.99% As_2O_3. However, high purity arsenates, arsenites, $AsCl_3$, $AsBr_3$ and other inorganic arsenic compounds are sold by chemical specialty companies. Electronic grade arsine (99.995 to 99.998% pure) may be used for calibration of instrumentation, or arsine may be generated from primary standard As_2O_3 and high-purity reagents. Analytical standards of arsenical pesticides are available from the U.S. Environmental Protection Agency (THOMPSON 1976). These standards are available at no charge to any legitimate laboratory engaged in residue analysis.

Care should be taken in preparing fortified samples from the substrates of interest and primary standards. The standard should be uniformly dispersed or dissolved in the matrix, a task that may be difficult with some water-soluble arsenicals and fatty tissues. The National Bureau of Standards (NBS) currently provides two organic reference materials which have been analyzed for arsenic. Only one of these, lyophilized orchard leaves (SRM 1571) is certified for arsenic (at 14 ± 2 $\mu g/g$). Bovine liver (SRM 1577) has been analyzed by NBS for arsenic (0.055 $\mu g/g$), but is not certified. It is also lyophilized. Other materials soon to be issued by NBS will be certified for arsenic (URIANO 1976). These include oyster meat (SRM 1566), spinach (SRM 1570), tomato (SRM 1573), wheat flour (SRM 1567), rice flour (SRM 1568), and pine needles (SRM 1575). Tuna fish will be issued as a research material only. These

NBS-certified materials will provide a very good basis for calibration of analytical recoveries and quantitative measurement of total arsenic in food and related biological substrates.

V. Methods of measurement

Most determinative methodology for arsenic is based on detection of atomic species or colored complexes prepared most often from arsine; hence, measurement is usually in terms of the total amount of arsenic in the sample. Colorimetric measurement has been used for arsenic residue analysis for many years and is still popular due to its low cost and simplicity. However, it is being rapidly replaced by atomic absorption spectrometric analysis. Other often-used methods are neutron activation analysis and gas chromatography. The latter is particularly valuable for differentiation between specific arsenic compounds.

a) Colorimetry

Three methods have received 'official final action' by the Association of Official Analytical Chemists (AOAC) and are recommended for determination or arsenic residues (Horwitz 1975). The first of them is the Gutzeit method, which dates back nearly 100 years (Gutzeit 1879) and is rarely used today. Although not classified as a colorimetric method by the AOAC, it is based on the formation of a brown color produced when arsine gas reacts with mercuric bromide-treated paper. Visual comparison of the color density produced from the sample with that produced from several standards of As_2O_3 is the basis of quantification. The molybdenum blue and silver diethyldithiocarbamate methods recommended by the AOAC are still in popular use. Both involve the production of highly colored As(III) complexes from arsine. In the former, arsine is absorbed in a solution of ammonium molybdate to give a blue color, which is measured spectrophotometrically at 845 nm; it is sensitive to 0.1 μg/g. The latter method uses a silver diethyldithiocarbamate (AgDDC) absorbent, which yields a red color (measured at 522 nm); it affords the best sensitivity (0.01 μg/g of As_2O_3) and is, therefore, the one most commonly used for residue analysis. A potential problem with the AgDDC method, however, is the possibility of interference from antimony, which produces a red complex with a maximum absorbance at 510 nm. Both this method and the molybdenum blue method can be applied to arsenic compounds separated from the sample by solvent extraction or co-precipitation, as well as by arsine generation.

Arnold *et al.* (1969) reported that both the molybdenum blue and the AgDDC colorimetric methods are subject to errors in the direction of underestimation of arsenic. They reported losses of up to 15% by either procedure, and found that slight variation in the acidity of the AgDDC solution can cause serious errors. The AgDDC method was found to be quicker and to require less analytical expertise for the determination of

arsenic in biological materials (*Analytical Methods Committee* 1975). However, precision was reported to be lower with the AgDDC procedure and the absorption maximum of the arsenic-DDC complex, which is of unknown composition, can range from 520 to 550 nm.

The color produced from the reaction of methylarsine with AgDDC is not the same as that produced from arsine. This difference was the basis of the simultaneous determination of the herbicide MSMA and arsenic acid in grass and cotton (LAKSO *et al.* 1973). The two hydrides were generated together, but measurements were made at 440 and 540 nm. Methylarsine was responsible for the shorter wavelength absorption. A two-color calculation was used to estimate MSMA and arsenic acid down to 0.2 and 0.01 μg/g, respectively.

Arsanilic acid (*p*-aminobenzenearsonic acid) in poultry and swine feeds is officially determined in the United States by a procedure based on color formation with ammonium sulfamate (HORWITZ 1975). Measurement is at 538 nm and detectability is in the μg range. The organoarsenical carbarsone (4-ureidophenylarsonic acid), also used in poultry feeds, can be determined by hydrolysis to arsanilic acid, diazotization, and coupling with *N*-1-naphthylethylenediamine dihydrochloride (*Analytical Methods Committee* 1971). The adduct is measured in water at 545 nm. The growth stimulant roxarsone was extracted from feed with two % $\cdot K_2HPO_4$ solution and basicified to pH 12 after cleanup to yield a bright yellow-orange color, which was measured at 410 nm (GEORGE and MORRISON 1971). Both methods are very insensitive and not suitable for trace analysis.

A recently developed colorimetric method is based on the action of arsenic(III) as an auxiliary catalyst in the osmium-catalyzed redox reaction of bromate-iodide (TARUMOTO and FREISER 1975). The absorbance is measured at 580 nm spectrophotometrically. The method was used to determine arsenic in human lung tissue at low μg/g levels.

Only a few other colorimetric methods for arsenic have been reported (TALMI and FELDMAN 1975). Although the use of these techniques appears to be declining, the low cost of their implementation still makes colorimetric methods attractive to many laboratories.

b) Atomic absorption spectrometry

A survey of the recent literature concerning the determination of arsenic in biological and environmental samples by atomic absorption spectrometry (AAS) indicates that this technology is rapidly replacing colorimetry as the methodology of choice. Most laboratories in the United States which are concerned with trace metal analyses are equipped for AAS. The method is very rapid and reliable. The only problem of note which is associated with the determination of arsenic by AAS is that the atomic lines of arsenic lie in the far ultraviolet region of the spectrum (189.0, 193.7, and 197.2 nm); therefore, sensitivity is poorer than it might otherwise be if absorption were at longer wavelengths.

In order to make atomic absorption measurements the sample must be atomized in the optical beam of the spectrometer. Monochromatic light (usually 193.7 nm) passing through the atomized sample is absorbed by arsenic atoms. The reduction in the radiation reaching the photomultiplier (compared to the reference beam in a double beam instrument) is detected and usually read out directly in terms of concentration. There are two major ways in which the sample may be atomized. In flame AAS it is aspirated directly into the flame, while in flameless AAS a heated graphite rod, furnace, or other atomizer is used. When organic solvent extraction is employed to recover arsenic from the sample, methyl isobutyl ketone has been found to be the most convenient for use for flame AAS. Often arsine gas is generated prior to AAS analysis and is introduced directly into the flame or atomizer. Both liquid and solid samples may be analyzed by the flameless carbon rod or furnace technique.

When standard flame techniques are used, the sensitivity achievable by AAS is poorer than that characteristic of some colorimetric methods. Under conditions in flames, atomic arsenic can be quickly converted to molecular As_2 and As_4, with resultant severe losses in sensitivity (Robinson *et al.* 1974). Absorption of wavelengths below 220 nm by acetylene in common air-acetylene flames limits the detection of arsenic to 0.25 to 1 μg/ml for aspirated solutions (Kahn and Schallis 1968, Hoover *et al.* 1974). Detection limits may be lowered by direct introduction of arsine into the flame. Holak (1969) reported a detection limit of 0.04 μg with an air-acetylene flame by trapping arsine with liquid nitrogen and then sweeping it directly into the flame at 50 ml/min.

Substitution of acetylene by other gases which do not absorb above 180 nm affords major improvements in sensitivity. The transmittance of an air-acetylene flame at 193.7 nm is only 19 to 28%, depending on the ratio of the two gases (Christian and Feldman 1970); thus, small variations in the mixture can have large effects on arsenic measurement. Dagnall *et al.* (1967) reported a two- to five-fold improvement in the detection limit for arsenic with nitrogen-hydrogen flames, which transmit about 42% of the light at 193.7 nm. Better results were obtained with an argon-hydrogen flame (%T = 82% at 193.7 nm), which permitted a detection limit of 0.05 μg/ml of arsenic (Kahn and Schallis 1968). A nitrogen-shielded, acetylene-nitrous oxide-fueled flame has been reported to be essentially transparent at 193.7 nm (Kirkbright and Ranson 1971); however, a detection limit of only one μg/ml was reported for arsenic with this flame.

Another approach which has been taken to improve the sensitivity of flame AAS for arsenic is through the use of quartz tube atomizers. This method can be used only in conjunction with arsine gas generation, since it involves passing the gas through a quartz cell mounted in the flame and optical path. Thompson and Thomerson (1974) reported a detection limit of 0.8 ng/ml for a soybean extract utilizing a special arsine generator and a silica atomizing tube mounted in an air-acetylene flame. Similarly,

SIEMER *et al.* (1976) were able to achieve a 0.3 ng detection limit for arsenic in dried orchard leaves using a hydrogen-oxygen flame. Both groups used conventional hollow-cathode lamps (HCLs) as radiation sources.

The flame-heated atomizer tube techniques cited above are essentially flameless AAS methods. In fact, some workers have dispensed with the flame and used electrically-heated quartz tubes (CHU *et al.* 1972, WAUCHOPE 1976). More usual methods of flameless AAS involve the heated graphite atomizer (HGA), carbon rod, or tantalum foil-lined graphite tube. The graphite furnace (HGA) and carbon rod atomizers are most often used for trace metal analysis by AAS (MASSMANN 1967). They offer much improved sensitivities and greater versatility for most applications. With these atomizers it is possible to analyze directly inorganic and organic solids, as well as liquids. Detection limits for trace metals range from 10^{-8} to 10^{-12} g and only very small sample sizes (one to 50 mg) are required. The furnace is heated in three programmed stages to effect drying (or solvent removal), ashing, and atomization. Temperatures for each stage may be varied. Since many As(III) compounds may suffer some loss during the ashing stage, sensitivity may be limited to one to ten ng by HGA (HANSEN 1971). However, addition of Ni^{2+} salts to the sample will decrease the volatility of arsenic (EDICER 1975). Arsenic has been found to be stable to charring temperatures up to 1,200°C when a large excess of nickel is added, enabling quantities of less than one ng to be determined.

Although it may be possible to analyze unashed solid samples by flameless AAS, the limited sample capacity may necessitate dissolution or homogenization in order to acquire a small but uniform sample. Hence, for many food samples and biological tissues, it is probably best to digest or ash the sample prior to analysis.

Another development which has added substantially to the improvement of AAS analysis for arsenic is the electrodeless discharge lamp (EDL). This radiation source was cited in the literature as long ago as 1968 as affording better sensitivity for arsenic than the conventional hollow cathode lamps (DAGNALL and WEST 1968). However, only recently have EDLs begun to replace HCLs for arsenic determinations. EDLs are very intense at the principal resonance lines and are free of carrier gas background; thus they overcome two of the major weakness of hollow cathode source lamps for elements such as arsenic which absorb in the vacuum ultraviolet region. Sensitivities for arsenic may be increased by two to ten times by use of the EDL (MENIS and RAINS 1969, BARNETT 1973, BARNETT *et al.* 1976). By using the combination of HGA (with Ni^{2+} matrix modification) and EDL, FREEMAN *et al.* (1976) were able to detect less than 50 pg of arsenic. They measured less than one ng of arsenic in undigested fish extracts without difficulty. Results and precision were equivalent for the undigested, wet ashed, and arsine generation methods. The authors also pointed out that the HGA is

particularly well suited for measuring trace amounts of arsenicals collected from a chromatographic column.

c) Radioactivation analysis

Neutron activation analysis (NAA) is frequently used by residue chemists for the determination of a broad spectrum of trace elements in biological and environmental samples. It has the advantage of being essentially nondestructive and the disadvantage of requiring a nuclear reactor or other neutron flux source. Trace elements may be determined in the untreated matrix, but often it is necessary to isolate those of interest before NAA is performed.

Arsenic has only one stable isotope, ^{75}As, which is converted in part to ^{76}As by neutron absorption. The half-life of ^{76}As is 26.5 hr and it is usually measured by γ-ray spectrometry, monitoring the most intense (559 keV) photopeak. Although the detection limit for arsenic by NAA may be as low as 0.1 ng (TALMI and BOSTICK 1975 a), this limit is difficult to achieve in practice because of interference from ^{24}Na and possibly other radionuclides. In order to eliminate interference from ^{24}Na ($t_{1/2} =$ 15 hr), the sample must be allowed to stand for three to nine days after activation (TALMI and FELDMAN 1975, LAMBERT 1976) or arsenic must be separated from the matrix by volatilization before or after activation. If the former approach is taken, problems may be encountered from the introduction of interfering contaminants. Another potential difficulty is interference from ^{82}Br and ^{122}Sb, which produce photopeaks at 554 and 564 keV, respectively. These elements codistill with arsenic when usual volatilization techniques are used (LAMBERT 1976) and have half-lives which are longer than that of arsenic. Measurement of the 559 keV photopeak of ^{76}As in the presence of ^{82}Br and ^{122}Sb is generally not difficult if high resolution detectors are used.

In order to determine levels of arsenic in human hair below 70 $\mu g/g$, ATALLA *et al.* (1965) had to fractionally distill the element as $AsCl_3$ from digested samples. Under the conditions of the distillation, $AsCl_3$ was recovered at 104°C, while $SbCl_3$ was distilled at 110°C. Samples with high arsenic levels could be analyzed without treatment. SAMSAHL (1967) determined arsenic along with bromine, antimony, mercury, and selenium in soft animal tissues by fractional distillation from H_2SO_4 and 30% H_2O_2 and subsequent ion-exchange chromatography. Limits of detection were not established. The method was said to be reliable for biological material containing zero to 20 mg of sodium chloride. THOMAS and KRISTENSEN (1968) found NAA valuable for determining arsenic residues in human kidney biopsies where only a few milligrams of sample is obtainable. They separated arsenic as arsine after activation and measured quantities as low as 1.6 $\mu g/g$ of tissue. Arsine generation was also the method of choice by DUKENBAEVA and SMIRNOV (1968), who determined arsenic in various biological organs, skin, and excretions. The samples were first mineralized, then activated and reduced to the hydride.

The $^{76}AsH_3$ was trapped on cotton wool impregnated with $HgCl_2$ for scintillation counting. LUNDE (1970) used molecular gel filtration and NAA to determine arsenic in various tissues of fish and shellfish. Levels as low as 0.1 μg/g were reported for one-g samples. The same technique was used by NORHEIM and STEINNES (1975) to determine protein-bound arsenic in human livers. Liver proteins were fractionated by passing buffered homogenates through a column of Sephadex® G-75 and the liquid fractions were sealed in quartz for activation. Three days were allowed to reduce ^{24}Na activity. Samples of dried animal tissues were activated with thermal neutrons by RAKOVIČ (1969), then subjected to simple precipitation separation to determine As residues as $^{76}As_2S_3$. About 1.4% of the activity of the precipitation was found to be due to co-precipitated ^{24}Na.

GEISMAN *et al.* (1969) used NAA to determine arsenic residues in tomatoes and various parts of the tomato plant. They simply dried the samples at 150°C for two hr, encapsulated them in polyethylene containers, and activated them. The detection limit was about 0.2 μg/g. Essentially the same technique has been employed for arsenic in grapes (DARVIS *et al.* 1970) and cigarettes (LEE and MURPHY 1969).

Neutron activation analysis was the method of choice for determination of arsenic levels in NBS standard reference materials 1571 (orchard leaves) and 1577 (bovine liver). The lyophilized materials were sealed in quartz vials for activation and were analyzed within a few hours; however, the 657 keV γ-ray photopeak of ^{76}As was selected to avoid interference (ORVINI *et al.* 1974).

OWENS and GLADNEY (1976) obtained results agreeing within the uncertainty of the data for arsenic in natural waters by NAA and flameless AAS. Precision appeared to be somewhat better for the flameless AAS data, however. Excellent agreement was reported by FIORINO *et al.* (1976) for arsenic determined in dried fish samples by NAA and flame AAS (arsine generation). LUNDE (1973 b), on the other hand, found x-ray fluorescence analysis to be quicker but less sensitive than NAA for As in fractionated fish extracts.

d) Other spectroscopic methods

Several other spectroscopic techniques have been found useful for determination of arsenic. These include x-ray fluorescence, optical or atomic emission, atomic fluorescence, and mass spectrometry. The first two methods have been applied to a substantial degree for arsenic determinations since they are popular techniques for multi-element analyses. Neither x-ray fluorescence nor optical emission spectroscopy would be usual methods of choice for determination of trace level of arsenic in biological specimens, however.

X-ray fluorescence is a truly nondestructive technique, but is relatively insensitive, and may require special sample treatment and long counting times. Instrumental costs are also high. For biological matrices, it is frequently necessary to isolate the arsenic residue, usually by co-

precipitation or solvent extraction. Lead has been cited as an interference (Reymont and DuBois 1971). Lunde (1973 b) reported sensitivity limits of two to five μg/g for x-ray fluorescent measurement of arsenic in marine biological material, depending on the matrix and other elements present. Dried, homogenized samples were pressed into tablet form with ten % wax for analysis. The detection limits were much poorer than those reported by Marcie (1967) for water samples (0.01 μg/g or less). Talmi and Bostick (1975 a) reported much better sensitivity by utilizing thionalide co-precipitation and nearly monochromatic radioactive sources. Detection of ten to 20 ng of arsenic with a one-hr counting time was achieved. Iron, copper, and sulfur were said to be among the few spectral interferences. Proton-induced x-ray emission analysis (PIXEA) has been employed to determine arsenic in fish and crabs (Fowler *et al.* 1975). Samples were freeze-dried for analysis. Detection limits of < 0.1 μg/g and < 0.2 μg/g were shown for fish and crabs, respectively.

Optical emission spectroscopy (OES) is generally slightly inferior to x-ray fluorescence in sensitivity and equipment costs are also high. Like x-ray fluorescence, its greatest value is in rapid, multi-element analysis. The most common instruments use a d.c. arc or high-voltage spark to vaporize the sample. The sample is usually dissolved in an electrolytically conductive liquid, such as strong mineral acid, and concentration may be necessary. Organic liquids and extracts may be volatilized without difficulty, however. With conventional hollow electrodes, arsenic may be completely volatilized in 15 sec (Loeske 1976). The rotating disk technique (Kopp and Kroner 1965, Kopp 1971) will permit longer burn times (30 to 60 sec) and thus enhance sensitivity for arsenic. The 235- and 229-nm lines are most frequently used in arc-source OES. The 189-nm line is the most intense atom line, but its wavelength is below the normally usable range (Ahrens and Taylor 1961), unless vacuum instrumentation is used. Braman *et al.* (1972) passed a d.c. discharge through an atmosphere of arsine contained in a sealed electrode assembly to detect approximately one ng of arsenic. The 228.8-nm emission line was monitored. Very substantial improvements in OES sensitivity can be achieved through the utilization of plasma sources for excitation (Dickenson and Fassel 1969). The higher source temperatures (9,000 to 10,000°C) minimize matrix effects and atomize higher proportions of the elements of interest. Detection limits down to 40 ng/ml have been reported for induction-coupled plasmas (Fassel and Kniseley 1974). Lichte and Skogerboe (1972) coupled an arsine generator to a microwave-induced argon plasma to determine < 3 ppb of arsenic in a one-ml sample. They found arsenic in blood at 0.22 to 52 μg/g, in hair at 0.23 μg/g, and in plant leaves at 9.5 μg/g.

Atomic fluorescence spectroscopy (AFS), which is usually performed on AAS equipment, suffers from the same problems of flame interference as flame AAS. However, since measurement is made at a 90° angle to the flame, sensitivity for As is several times better with AFS (Morrison 1965). Using an EDL light source and a hydrogen-nitrogen flame, Dag-

NALL *et al.* (1968) were able to detect 0.1 μg/g of arsenic in solution. They monitored the 189-nm line. Nonflame AFS may provide still better sensitivity, but less than flameless AAS; hence, there has been much less use of AFS than AAS for arsenic analysis.

A new flame technique known as molecular emission cavity analysis (MECA) has been reported to detect less than 0.1 μg/ml of arsenic (BELCHER *et al.* 1974). The sample is placed within a small cavity in the end of a rod, which is then positioned in a hydrogen-nitrogen flame. Copper and silver were reported to be interferences.

Mass spectrometry is not strictly a spectroscopic technique, but will be discussed here for convenience. The spark-source mass spectrometer (SSMS) is a tool of relatively recent application (BROWN *et al.* 1972). Like x-ray fluorescence and optical emission spectroscopy, SSMS is basically for survey purposes, and is not particularly suited for single-element measurements. The instrumentation is also very expensive, requires highly skilled operators, and analysis times are somewhat lengthy. As^+ and As^{2+} ions are produced by a high-intensity spark and identified after electrostatic and magnetic focusing at m/e 75 and m/e 37.5. Calcium, potassium, chlorides, and bromides are potential interferences. Organic compounds which survive the spark may also interfere; hence, samples usually must be ashed before analysis. The absolute detection limit for arsenic by SSMS is about 15 ng. Sample size is limited to 5 to 50 mg. SSMS has greater value as a qualitative instrument rather than one for quantitative determination, especially for arsenic, which is one of 12 elements not amenable to the isotope-dilution technique of measurement. Precision averages about 20% RSD and accuracy (against NBS standards) ranges from 30 to 50% (DAUGHTREY 1976).

Volatile organoarsenicals (e.g., methyl and phenyl arsines) can be readily analyzed by conventional electron impact mass spectrometry. This is most frequently performed in conjunction with gas chromatography and will be discussed later. One particularly valuable technique for indentification of nonvolatile or thermally unstable organoarsenicals is field desorption mass spectrometry (BECKEY 1969). In this method, molecules are ionized by the application of high voltage and are desorbed from an emitter (e.g., carbon dendrites) into the mass spectrometer. Only very small sample sizes are required (10^{-8} to 10^{-9} g) and since predominately molecular ions are produced, sample cleanup can often be eliminated. Cacodylic acid, which can not be easily gas chromatographed and is otherwise difficult to analyze by MS, can be readily determined by FDMS (RYAN *et al.* 1975). Quantitative information is difficult to obtain by FDMS, however, especially for compounds with only one stable isotope.

e) Chromatography

Various chromatographic techniques have been applied to the separation for subsequent determination of both organic and inorganic arseni-

cals. Ion-exchange chromatography can be utilized to separate As(III) and As(V) compounds from each other and from organoarsenicals (LUNDE 1973 a). VON ENDT *et al.* (1968) separated MSMA and inorganic arsenate and arsenite by thin-layer chromatography (TLC). Paper chromatography (PC) and TLC have been applied to separate cacodylic acid, arsenate, and arsenite extracted from plant tissue (SACHS *et al.* 1970). TLC was also effective for estimation of arsanilic acid in animal feed (CIERI 1976). Measurements by PC or TLC are usually made visually after spraying with color-producing reagents (e.g., ammonium molybdate or *p*-dimethylaminobenzaldehyde) and are, therefore, only semiquantitative. *In situ* spectrometric scanning or spot-removal for extraction can be carried out for more quantitative measurements.

The problems of quantification may be overcome by gas chromatography (GC). Volatile arsenicals can be determined in very small quantities with high precision and accuracy by GC with detectors such as flame ionization (FID), alkali flame ionization (AFID), flame photometry (FPD), electron capture (ECD), and microwave emission (MED). Detection limits are in the pg-to-ng range.

The total arsenic content of biological materials can be readily determined by conversion to volatile inorganic or organic arsenic compounds, which can then be gas chromatographed. As early as 1964, $AsCl_3$ had been separated from other metal halides by GC (TADMOR 1964). Iododimethylarsine is another arsenical which can be readily gas chromatographed and detected by electron capture (SODERQUIST *et al.* 1974). DDC complexes of arsenic have also been determined by GC-ECD (DAUGHTREY *et al.* 1975). Triphenylarsine is the derivative most often prepared for total arsenic analysis by GC. SCHWEDT and RÜSSEL (1972) were the first to use the methodology. They reacted arsenic-DDC with diphenylmagnesium Grignard reagent and chromatographed the Ph_3As at 220°C on a 3-mm(id) × 2-m glass column packed with 5% Carbowax® 20M on Gas Chrom® Q. Using an FID, they could detect 0.4 ng, which permitted the determination of total arsenic levels in biological media as low as two μg/g. TALMI and NORVELL (1975) later used basically the same method as SCHWEDT and RÜSSEL (1972) to determine total arsenic and antimony simultaneously in orchard leaves and other environmental samples. They used a microwave emission spectrometric detector, with which they could detect 20 pg of Ph_3As (corresponding to 30 ng/g of total arsenic). A 91-cm long quartz capillary column (0.5-mm id) packed with four % FFAP on Gas Chrom Q was used. Problems with accumulation of nonvolatile residues on the column with resultant loss of sensitivity and precision were cited.

LÜSSI-SCHLATTER and BRANDENBERGER (1974) achieved pg-level sensitivity for arsenic by gas chromatography-mass spectrometry. Arsine was generated and determined by specific ion MS, using germanium hydride as an internal standard.

Gas chromatography has also been used to determine specific

arsenic compounds in biological samples, either directly or after derivatization. WESTON *et al.* (1971) analyzed animal feeds for arsanilic acid and carbarsone by reduction to aniline, which was then determined by GC-FID. Methanearsonic acid, which is the free acid of the herbicides MSMA and DSMA, was converted to a ethylene glycol chelate, $CH_3As\begin{bmatrix}-OCH_2\\ |\\ -OCH_2\end{bmatrix}_2$, for GC analysis at 140° C on 10% OV-17 (JOHNSON *et al.* 1972). Microwave emission detection provided a minimum detectable limit of 1.5 ng/sec and a linear range of one to ten μg. Better sensitivity was obtained with an AFID, but linearity and reproducibility were lacking. As previously mentioned, cacodylic acid can be converted to $(CH_3)_2AsI$, which can then be determined by GC-ECD on 10% DC-200 at 125°C (SODERQUIST *et al.* 1974). Inorganic and mono- and dimethylarsenicals were individually determined in urine and water as their corresponding DDC complexes. Electron capture detection permitted determination of 73 ng/ml of arsenic diethyldithiocarbamate and 15 ng/ml and 40 ng/ml, respectively, of the mono- and dimethyl derivatives. Chromatography was on five % OV-17 at 80° to 170°C. TALMI and BOSTICK (1975 a) chose to reduce the alkylarsenic acids MMAA and DMMA to methylarsine and dimethylarsine, respectively, for GC-MED analysis. Chromatography on five % Carbowax 20M on Chromasorb® 101 at 175°C provided an arsenic detection limit of 20 pg. Trimethylsilyl derivatives of arsenate, arsenite, cacolylate, and methanearsonate salts have been gas chromatographed (BUTTS and RAINEY 1971, SODERQUIST *et al.* 1974), but are difficult to work with because of their hydrolytic instability. Cyanoethyl derivatives have also been investigated for GC analysis, but their synthesis was regarded as too slow for practical application (SODERQUIST *et al.* 1974).

f) Electrochemical methods

Polarography has been used for a number of years for arsenic analysis in various biological materials (ARNOLD and JOHNSON 1969, TALMI and FELDMAN 1975). Differential pulse polarography (MEYERS and OSTERYOUNG 1973) and single-sweep polarography (WHITNACK and BROPHY 1969) are several orders of magnitude more sensitive than conventional polarography and may be used to determine sub-microgram amounts of arsenic. Only arsenite can be measured polarographically, as the method depends on oxidation of As(III) to As(V) for detection. Lead, tin, and thallium can result in serious interferences if present in quantities approximating that of arsenic (MEYERS and OSTERYOUNG 1973). REINKE *et al.* (1975) successfully applied differential pulsed polarography to the separate determination of arsenite and arsenate in fish and shellfish tissues. Arsenite was selectively extracted into benzene from the tissues as $AsCl_3$ following digestion in HCl. Partitioning into water allowed polarographic measurement. The arsenate remaining in the tissues was reduced to arsenite with cuprous chloride and subsequently analyzed.

Amperometric and coulometric titrametric techniques have been used to measure arsenic recovered from organic matter (SANDU *et al.* 1973, SIMON *et al.* 1968); the sensitivities of the methods, particularly the former, limit their utility for trace analysis. Coulometric titration with amperometric end point detection was found to be convenient for measurement of arsenic concentrations between 10^{-7} and $10^{-3}M$ (BRUCKENSTEIN and JOHNSON 1964).

Anodic stripping voltammetry (ASV), which has been demonstrated to be a valuable technique for trace metal analysis in foods (ZINK and ASKEW 1975), has recently been applied to measurement of arsenic (ANONYMOUS 1976). The method requires a special composite gold-graphite electrode which is capable of plating out arsenic for detection at μg/g concentrations. As for most other electrochemical techniques, all arsenic must be reduced to the +3 state for measurement. Copper in amounts over one μg seriously interferes with the analysis. Bi, Sb, and Sn are also potential interferences. ASV has been evaluated for arsenic in lyophilized orchard leaves (ANON. 1976).

Although perhaps better classified as a chromatographic technique, electrophoresis can be used to separate and detect arsenic compounds in biological systems. EDWARDS *et al.* (1975) determined the sodium salts of arsenic, arsenious, methylarsonic, and cacodylic acids by electrophoresis on cellulose TLC plates. After separation, the plates were developed sequentially with ammonium sulfide, Jüngnickel's Reagent A (JÜNGNICKEL 1967), and short-wavelength ultraviolet light, which produced different colored spots corresponding to each ion. Minimum detectable limits were 0.1, 0.5, 1.5, and 2.0 μg, respectively, for arsenate, arsenite, methylarsonate, and cacodylate ions. The method was said to be simple, rapid, sensitive, and inexpensive.

Summary

Sampling of foods and biological specimens can present a greater challenge with respect to procuring representative samples than in the case of environmental media, such as water, air, and soil. Not all portions of plant and animal food products are intended for human consumption, and the composition of that which is consumed may be altered by cooking or other preparation prior to consumption. Collection and sampling of biological materials, therefore, require careful planning.

Once the best representative sample is selected and homogenized, the course of analysis chosen will depend on the information sought. For total arsenic determination, it is generally best to first decompose the biological matrix by low-temperature ashing or wet digestion. If the lipid content is high, wet digestion may present problems. By either method, volatile arsenic compounds may be lost unless special precautions are taken. Juices and other low-fat liquids may be analyzed directly or may be adaptable to solvent extraction techniques for isolation of arsenic.

Atomic absorption spectrometry using non-flame atomization techniques and arsenic electrodeless discharge lamps appears to be the best methodology for total arsenic determination. Identification and analysis of specific arsenic compounds are somewhat more complicated. Separation of the compounds from the substrate without alteration, which is usually required, may be a difficult task for high-lipid and high-protein tissues. Specific isolation techniques and gas chromatography are most effective for compound identification and measurement. Field desorption mass spectrometry is particularly valuable for identification of specific organoarsenicals, but is too sophisticated and costly for general application.

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